An Open-source IVIVE Workflow Integrating QSAR and PK Models

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Introduction

- In vitro high-throughput screening (HTS) assays can accelerate identification of potentially toxic chemicals. However, before in vitro HTS assays can be used in risk assessment, the ability of in vitro activity to predict in vivo dose-response relationships needs to be evaluated.

- In vitro to in vivo extrapolation (IVIVE) links in vitro assay results to in vivo effects by estimating the daily in vivo equivalent administered dose (EAD) expected to produce a plasma or tissue concentration equivalent to an in vitro effective concentration (**Figure 1**).

- Proprietary software has typically been used for predicting pharmacokinetic (PK) parameters and PK modeling. However, open-source software may be better suited for regulatory applications due to increased transparency.

- To facilitate IVIVE analysis with open-source tools, we developed an IVIVE workflow incorporating transparent quantitative structure-activity relationships (QSARs) to parameterize PK models of varying complexity.

Figure 1. A Reverse Pharmacokinetic Model for In Vitro to In Vivo Extrapolation
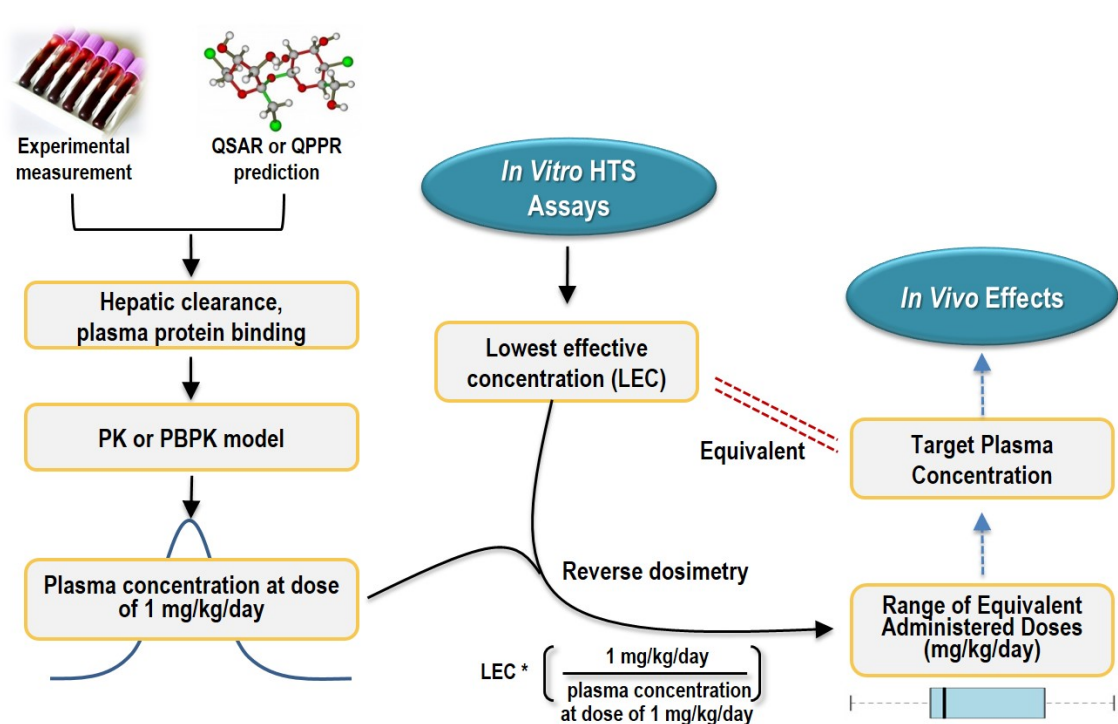
Development of Open-source IVIVE Workflows

- **Figure 2** shows the scheme of an IVIVE workflow we built using Konstanz Information Miner (KNIME). The workflow incorporates:
    - A one-compartment population-based PK (P-PK) model (**Figure 3A**) that incorporates Monte Carlo simulation to cover physical variability across individuals (Wetmore et al. 2012)
    - A generalized three-compartment physiologically based pharmacokinetic (PBPK) model (**Figure 3B**; ongoing work)

    Both models predict a daily EAD that would lead to a blood concentration equivalent to the effective concentration from an in vitro assay.

- Required workflow inputs include:
    - In vitro activity, which can be expressed as lowest effective concentration (LEC), half-maximal activity concentration (AC50), activity concentration at cutoff (ACC), or other concentration metric
    - PK parameters, including fraction of chemical unbound to protein (fu), intrinsic clearance, renal clearance for the P-PK model, and partition coefficients (PCs) between plasma and gut, kidney, and liver for the generalized PBPK model

- The workflow output is an EAD value, which can be compared to user-provided in vivo lowest effective levels (LELs) or human exposure values.

- The workflow provides values for both EAD_Adj, the daily equivalent dose that results in free chemical concentrations in the blood equivalent to corresponding ACCs, and EAD_Total, the daily equivalent dose that results in total chemical concentrations in the blood equivalent to corresponding ACCs.
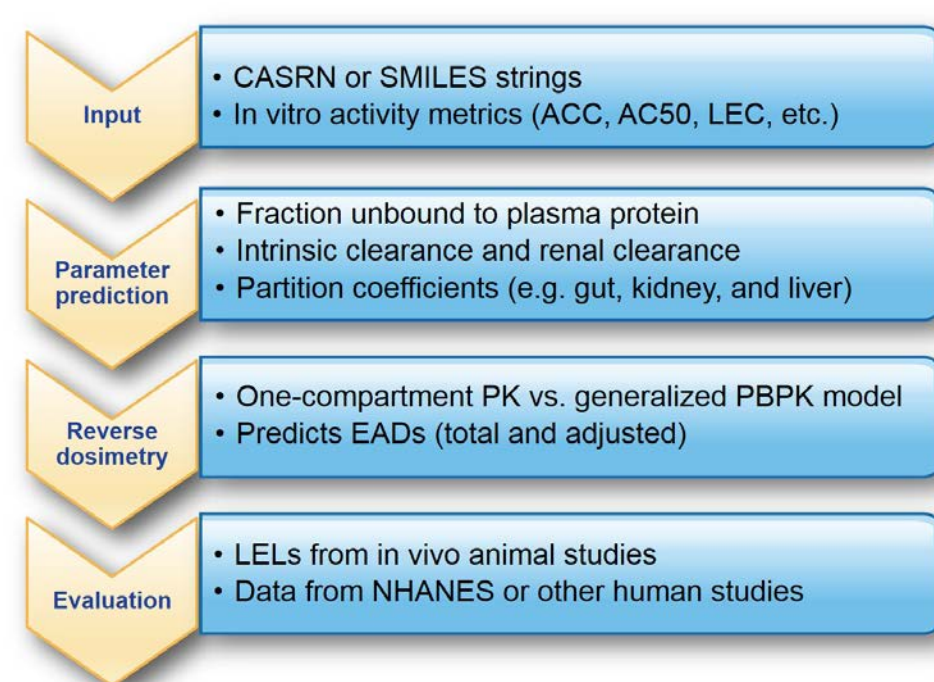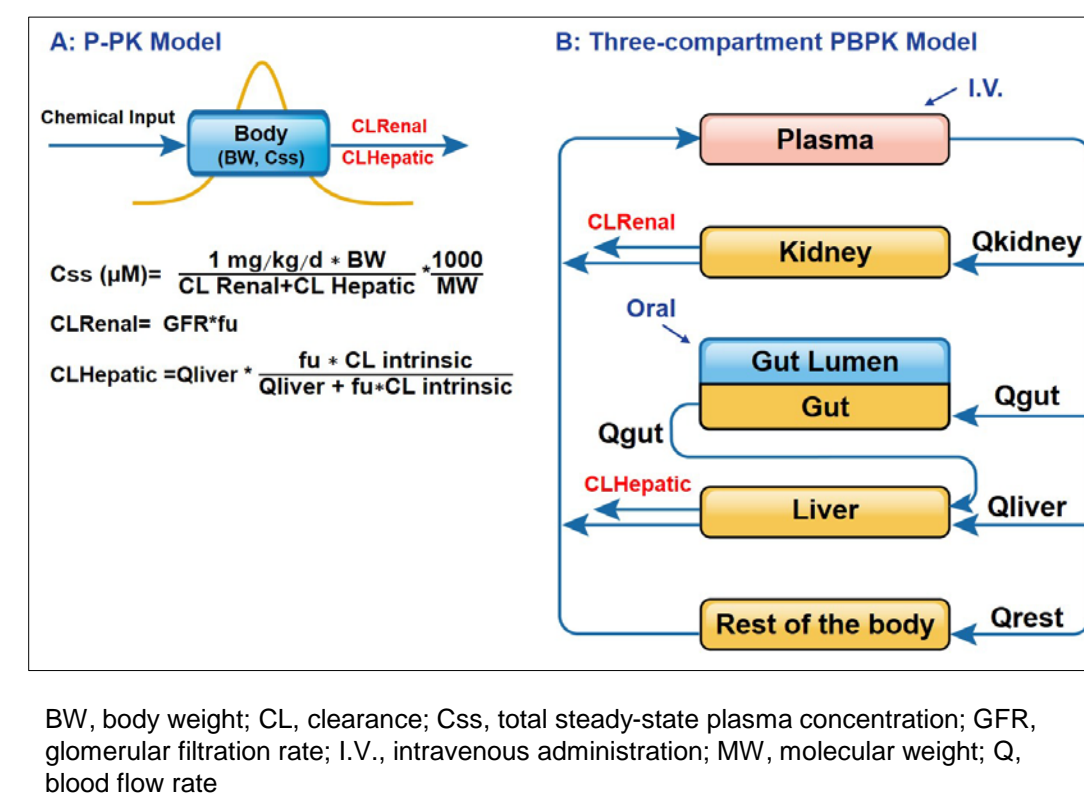
Figure 2. Scheme of IVIVE Workflow

Figure 3. Structure of Pharmacokinetic Models

BW, body weight; CL, clearance; Css, total steady-state plasma concentration; GFR, glomerular filtration rate; I.V., intravenous administration; MW, molecular weight; Q, blood flow rate

Development of QSAR Models to Predict Input PK Parameters

- We built QSAR models to predict input PK parameters: fu, Henry's law constant (HLC), and PCs between plasma and gut, kidney, and liver.
    - HLC is the proportionality factor of Henry's law, which states that the amount of dissolved gas is proportional to its partial pressures in the gas phase. HLC is used to calculate water:air partition coefficient.

- Binary molecular fingerprints were generated from the PaDEL program (Yap et al. 2011). A genetic algorithm was employed to select the most information-rich subset of fingerprint bits. Two machine learning approaches, multiple linear regression and support vector regression, were used to create the QSAR models.

- Experimental datasets were curated for each parameter, ranging from 69 -1808 chemicals. The models were trained on 75-80% of the data, and 20-25% of the chemicals were used as external test sets.

- The fu and HLC QSAR models were used in combination with an open-source logP model, which predicts the ratio of the concentration between water and octanol (Zang et al. 2017), to predict intrinsic clearance based on a published quantitative property-property relationship (QPPR) model (Kirman et al. 2015).

- **Figures 4-6** show that our models accurately predicted PK parameters (R^2 ranged from 0.79 to 0.86 between the predicted and experimental values for the external test sets). Where available, we compared performance of our models to that of ADMET Predictor software (Simulations Plus, Inc.), a proprietary software that uses QSAR models to predict biochemical, biophysical, and pharmacokinetic properties. Our models consistently performed better.
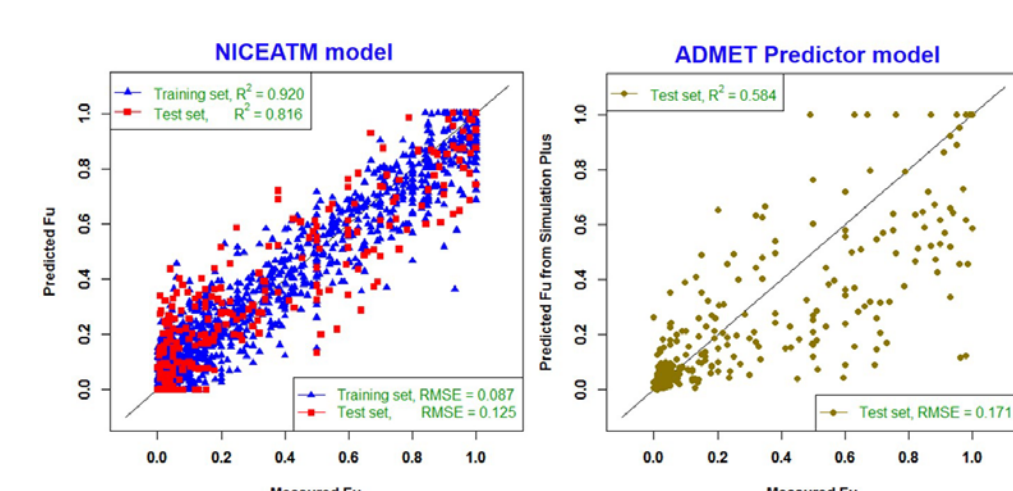
Figure 4. QSAR Models for Predicting fu

Figure 5. QSAR Models for Predicting Henry's Law Constant
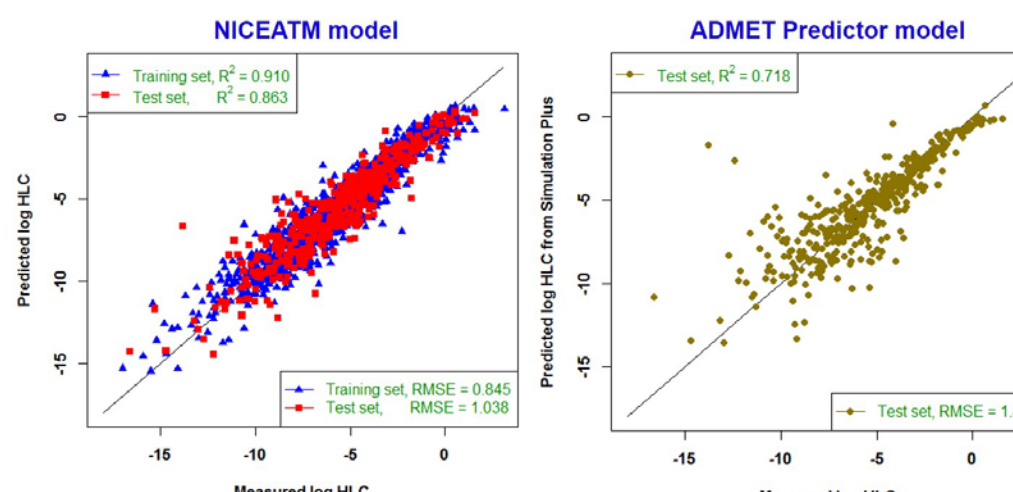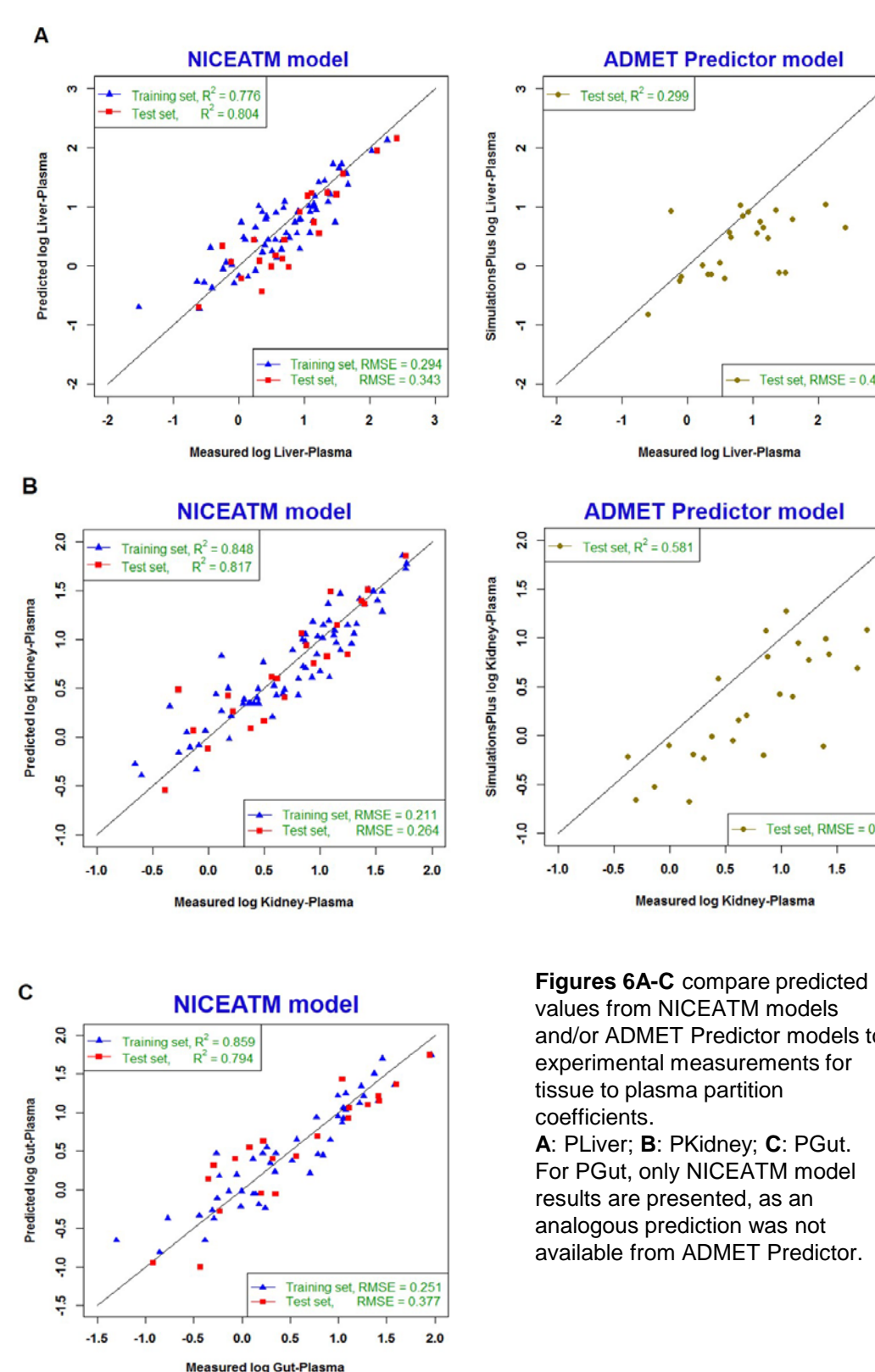

## Figure 6. QSAR Models for Predicting Tissue to Plasma Partition Coefficients

Figures 6A-C compare predicted values from NICEATM models and/or ADMET Predictor models to experimental measurements for tissue to plasma partition coefficients.
A: PLiver; **B**: PKidney; **C**: PGut. For PGut, only NICEATM model results are presented, as an analogous prediction was not available from ADMET Predictor.

Application of the Workflow

- We used the KNIME workflow incorporating a one-compartment P-PK model (**Figure 7**) to carry out IVIVE analyses on 32 potential estrogen receptor (ER) agonists.
    - Model parameters:
        - Predicted values for fu, logP, and HLC from our open-source QSAR models were used to calculate intrinsic clearance for each chemical.
        - In vitro ACC values from HTS assays targeted to each step of the ER agonist pathway were obtained from the ToxCast October 2015 release (Judson et al. 2015).
        - In vivo LELs from guideline-like injection uterotrophic studies were obtained from a published curated database (Kleinstreuer et al. 2016).
    - Workflow output:
        - EAD_Adj, the daily equivalent dose that results in free chemical concentrations in the blood equivalent to ER pathway ACCs
        - EAD_Total, the daily equivalent dose that results in total chemical concentrations in the blood equivalent to ER pathway ACCs

- **Figure 8** shows that EAD_Adj (red boxplot) predicted in vivo uterotrophic LELs more accurately, and EAD_Total (green boxplot) provided more conservative estimates of uterotrophic LELs. The range of EAD_Adj estimates overlapped the range of LELs in injection uterotrophic studies for 26 of 32 chemicals.

Figure 7. KNIME Workflow Incorporating a P-PK Model
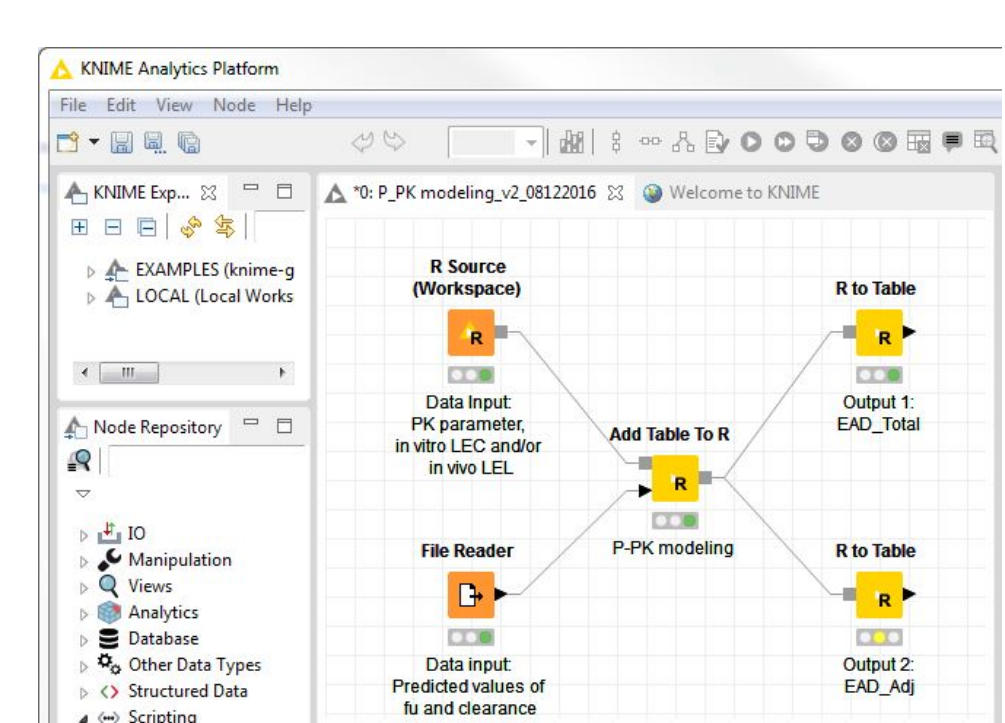
The KNIME workflow uses five modules: two input modules, one modeling module containing the P-PK model, and two output modules. The user executes each module by clicking the module tab. The bar under each module tab shows the execution status.

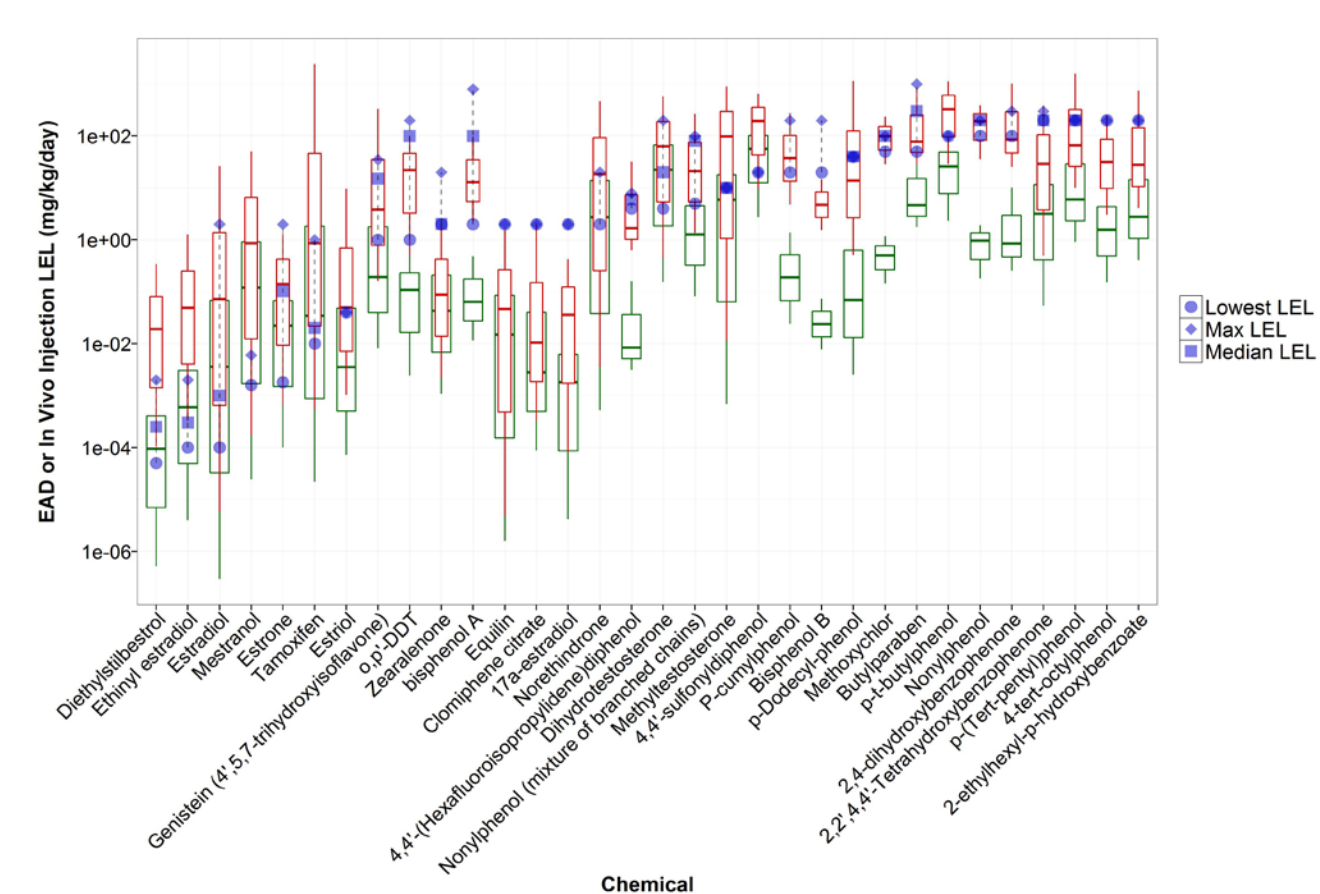## Figure 8. IVIVE Analysis (P-PK model): ER Pathway Assays Compared to Uterotrophic LELs

Figure 8 compares estimated EAD_Adj (mg/kg/day; red boxplots) and EAD_Total (mg/kg/day; green boxplots) predicted from ToxCast ER pathway assays using our IVIVE workflow to lowest, median, and maximum in vivo LELs (mg/kg/day; blue symbols and dashed lines) from injection uterotrophic studies.

Discussion and Conclusion

- Our open-source, ready-to-use workflows provide a transparent and easily accessible approach for IVIVE analysis. They can be used to study relevant toxicity potential for thousands of chemicals lacking in vivo data, expediting the safety assessment process.

- Predictions of input parameters for PK or PBPK models generated by our QSAR models correlated well with measured data, and our models generated better predictions than proprietary software for all the parameters.

- When evaluating estrogenic activities, the range of EAD estimates produced by the workflow incorporating the P-PK model correlated well with the range of in vivo uterotrophic LELs for the majority of chemicals tested. This result suggests that this IVIVE approach provides valid estimates of in vivo estrogenic activity from in vitro ER pathway assays.

- Ongoing work is focused on developing generalized PBPK models that incorporate other metabolic pathways, such as glucuronidation. Such models will be included in future versions of the KNIME workflow to improve prediction accuracy for phenolic compounds, for example.

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